PPARδ Inhibits UVB-Induced Secretion of MMP-1 through MKP-7-Mediated Suppression of JNK Signaling

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In the present study, we investigated the role of peroxisome proliferator–activated receptor (PPAR) δ in modulating matrix-degrading metalloproteinases and other mechanisms underlying photoaging processes in the skin. In human dermal fibroblasts (HDFs), activation of PPARδ by its specific ligand GW501516 markedly attenuated UVB-induced secretion of matrix metalloproteinase (MMP)-1, concomitant with decreased generation of reactive oxygen species. These effects were significantly reduced in the presence of PPARδ small interfering RNA and GSK0660. Furthermore, c-Jun N-terminal kinase (JNK), but not p38 or extracellular signal–regulated kinase, mediated PPARδ-dependent inhibition of MMP-1 secretion in HDFs exposed to UVB. PPARδ-mediated messenger RNA stabilization of mitogen-activated protein kinase phosphatase (MKP)-7 was responsible for the GW501516-mediated inhibition of JNK signaling. Inhibition of UVB-induced secretion of MMP-1 by PPARδ was associated with the restoration of types I and III collagen to levels approaching those in cells not exposed to UVB. Finally, in HR-1 hairless mice exposed to UVB, administration of GW501516 significantly reduced wrinkle formation and skin thickness, downregulated MMP-1 and JNK phosphorylation, and restored the levels of MKP-7, types I and III collagen. These results suggest that PPARδ-mediated inhibition of MMP-1 secretion prevents some effects of photoaging and maintains the integrity of skin by inhibiting the degradation of the collagenous extracellular matrix.

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INTRODUCTION

Peroxisome proliferator–activated receptor (PPAR) δ is a ligand-inducible transcription factor that modulates multiple biological functions pertaining to skin homeostasis (Sertznig *et al.*, 2008). PPARδ is abundantly and ubiquitously expressed in a variety of cell lineages, including skin-derived cells, and has been implicated in diverse aspects of skin physiology (Sertznig *et al.*, 2008). Recent studies have reported that PPARδ exerts anti-tumorigenic effects by regulating differentiation and proliferation in the skin (Borland *et al.*, 2008; Bility *et al.*, 2010). It has also been postulated that PPARδ is a key mediator of epidermal and dermal aspects of the wound-healing process: it converts extracellular inflammatory signals into organized patterns of gene expression, which eventually leads to the survival, migration, and differentiation of keratinocytes (Tan *et al.*, 2001; Di-Poï *et al.*, 2002). In fact, ligand-activated PPARδ promotes wound healing by upregulating TGF-β1-mediated expression of the extracellular matrix proteins in human keratinocytes and fibroblasts (Ham *et al.* 2010). Furthermore, we have recently demonstrated that ligand-activated PPARδ confers resistance to UVB-induced cellular senescence by upregulating PTEN, thereby modulating PI3K/Akt signaling and ultimately reducing generation of reactive oxygen species (ROS) in keratinocytes (Ham *et al.*, 2012). On the basis of its beneficial properties, including the acceleration of wound healing and the regulation of inflammatory responses and cellular senescence (Di-Poï *et al.*, 2002; Tan *et al.*, 2005; Ham *et al.*, 2010, 2012), PPARδ is a promising target for the treatment of skin disorders. However, the therapeutic efficacy of PPARδ ligands against skin disorders has not been fully elucidated (Sertznig *et al.*, 2008).

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that have pivotal roles in the degradation of the basement membrane (Visse and Nagase 2003). These enzymes are secreted in response to various stimuli, including UV light, oxidative stress, and cytokines, in a variety of cell lineages, including keratinocytes and dermal fibroblasts (Sárdy, 2009). UV-induced secretion of MMPs by skin cells is responsible for the degradation of dermal collagen

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Abbreviations: ERK, extracellular signal–regulated kinase; HDF, human dermal fibroblast; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MKP, mitogen-activated protein kinase phosphatase; MMP, matrix metalloproteinase; mRNA, messenger RNA; NAC, N-acetyl-L-cysteine; PPAR, peroxisome proliferator–activated receptor; ROS, reactive oxygen species; siRNA, small interfering RNA

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during the aging of human skin (Brennan *et al.*, 2003). Excessive levels of MMPs can impair the structural integrity of the epidermis and the dermis, resulting in photoaging, which is characterized by increased coarse wrinkles, thickening, roughness, and irregular pigmentation (Kligman and Kligman, 1986; Ma *et al.*, 2001; Jenkins, 2002; Rittié and Fisher 2002). In particular, elevated levels of MMP-1, MMP-3, and MMP-9 are demonstrated in the UV-radiated skin with degradation of collagen and elastin (Fisher *et al.*, 1997). Upon UV exposure, the generation of ROS in skin has a substantial role in degrading interstitial collagen through the activation and upregulation of matrix-degrading metalloproteinases (Brenneisen *et al.*, 1998; Yoon *et al.*, 2011). In addition, UV irradiation of human skin activates the mitogen-activated protein (MAP) kinase signal transduction pathways, including c-jun N-terminal kinases (JNK) and p38, which induce subsequent expression and activation of MMPs; thus, MAP kinase signaling cascades are involved in UV-induced MMP secretion (Fisher *et al.*, 1998). At present, MMPs are recognized as the primary enzymes responsible for the degradation of extracellular matrix proteins, such as collagen, fibronectin, elastin, and proteoglycan (Iddamalgoda *et al.*, 2008). Damage to collagen, among all the extracellular matrix molecules, is the primary factor in wrinkle formation associated with skin photoaging (Moloney *et al.*, 1992). Several studies have shown that the levels of MMPs are significantly increased in UV-irradiated skin (Fisher *et al.*, 1996, 1997; Lahmann *et al.*, 2001). In fact, UVB-mediated production of ROS increases MMP secretion in human dermal fibroblasts (HDFs) (Brenneisen *et al.*, 2002). Therefore, blockade of MMPs may represent one strategy for preventing UV-initiated photodamage caused by a complex cascade of biochemical reactions in the skin.

Here, we attempted to elucidate the role of PPARδ in UV-induced cellular damage using HDFs, in which we manipulated the activation and expression of PPARδ. Our results demonstrate that ligand-activated PPARδ attenuates the UVB-induced secretion of MMP-1 by inhibiting ROS generation, in a process mediated by the JNK/MKP-7 signaling pathway.

RESULTS

PPARδ inhibits UVB-induced secretion of MMP-1

As secretion of MMP-1 results from repeated exposure to UVB light (Dong *et al.*, 2008), we examined whether ligand-activated PPARδ affects the secretion of MMP-1 in HDFs exposed to UVB. When HDFs were exposed to UVB, we observed a marked increase in the secretion of MMP-1 and MMP-3 (Supplementary Figure S1a and b online). These increases were significantly suppressed in the presence of GW501516, a specific ligand for PPARδ (Figure 1a and b). In addition, GW501516 inhibited the UVB-induced upregulation of MMP-1 expression, suggesting the involvement of PPARδ in the inhibition of UVB-induced secretion and the expression of MMP-1 (Supplementary Figure S2 online).

To verify the role of PPARδ in blocking UVB-induced MMP-1 secretion, we examined the effect of GW501516 in cells treated with a small interfering (si)RNA against PPARδ. The level of PPARδ in HDFs was markedly reduced upon

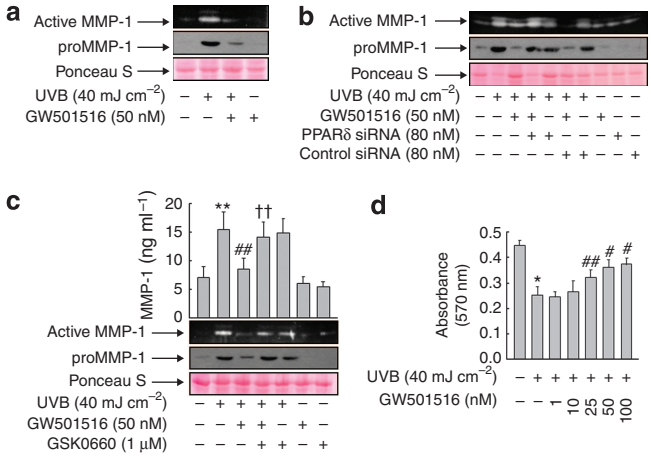

Figure 1. Peroxisome proliferator–activated receptor (PPAR) δ inhibits UVB-induced secretion of matrix metalloproteinase (MMP)-1. (**a**–**c**) Human dermal fibroblasts transfected without (**a**) or with (**b**) small interfering RNA (siRNA) or pretreated with GSK0660 for 30 minutes (**c**) were treated with GW501516 for 24 hours and then exposed to UVB. After incubation for 48 hours, conditioned media were subjected to ELISA, zymography, or immunoblot analysis. (**d**) Cells pretreated with GW501516 for 24 hours were exposed to UVB. Following incubation for 24 hours, a methyl thiazolyl tetrazolium assay was performed. GW501516 and GSK0660 were dissolved in DMSO. Results are expressed as the means ± SD ($n = 6$). $^{*}P<0.01$, $^{**}P<0.05$ versus untreated group; $^{\#}P<0.01$, $^{\#\#}P<0.05$ versus UVB-exposed group; $^{\dagger\dagger}P<0.05$ versus UVB plus GW501516-treated group.

transfection with PPARδ siRNA, whereas control siRNA, comprising a pool of nonspecific sequences, had no effect on PPARδ levels (Supplementary Figure S3 online). As expected, PPARδ siRNA, but not control siRNA, significantly suppressed the GW501516-mediated inhibition of MMP-1 secretion induced by UVB irradiation (Figure 1b). In addition, pretreatment of GSK0660, an antagonist for PPARδ, also reversed the effect of GW501516 on the MMP-1 secretion (Figure 1c).

To determine the role of PPARδ in UVB-induced cellular damage in HDFs, we next assessed the effect of GW501516 on the cell viability of HDFs exposed to UVB. Exposure of HDFs to UVB resulted in a marked decline in cell viability, as assessed by the methyl thiazolyl tetrazolium assay. In the presence of GW501516, however, UVB-mediated cellular damage was significantly attenuated in a dose-dependent manner (Figure 1d).

PPARδ suppresses ROS generation induced by UVB radiation

As UVB induces ROS generation in HDFs (Bossi *et al.*, 2008), we examined the effects of GW501516 on ROS production in HDFs exposed to UVB. Whereas UVB irradiation significantly increased ROS generation, pretreatment with GW501516 significantly suppressed UVB-induced ROS production (Figure 2a and b). The reduction in ROS generation upon treatment with GW501516 was not observed in cells transfected with PPARδ siRNA or pretreated with GSK0660, suggesting that the effect of GW501516 on ROS production is dependent on PPARδ (Figure 2a and b). Furthermore, pretreatment with *N*-acetyl-L-cysteine (NAC), a thiol antioxidant, also caused a reduction in UVB-induced ROS

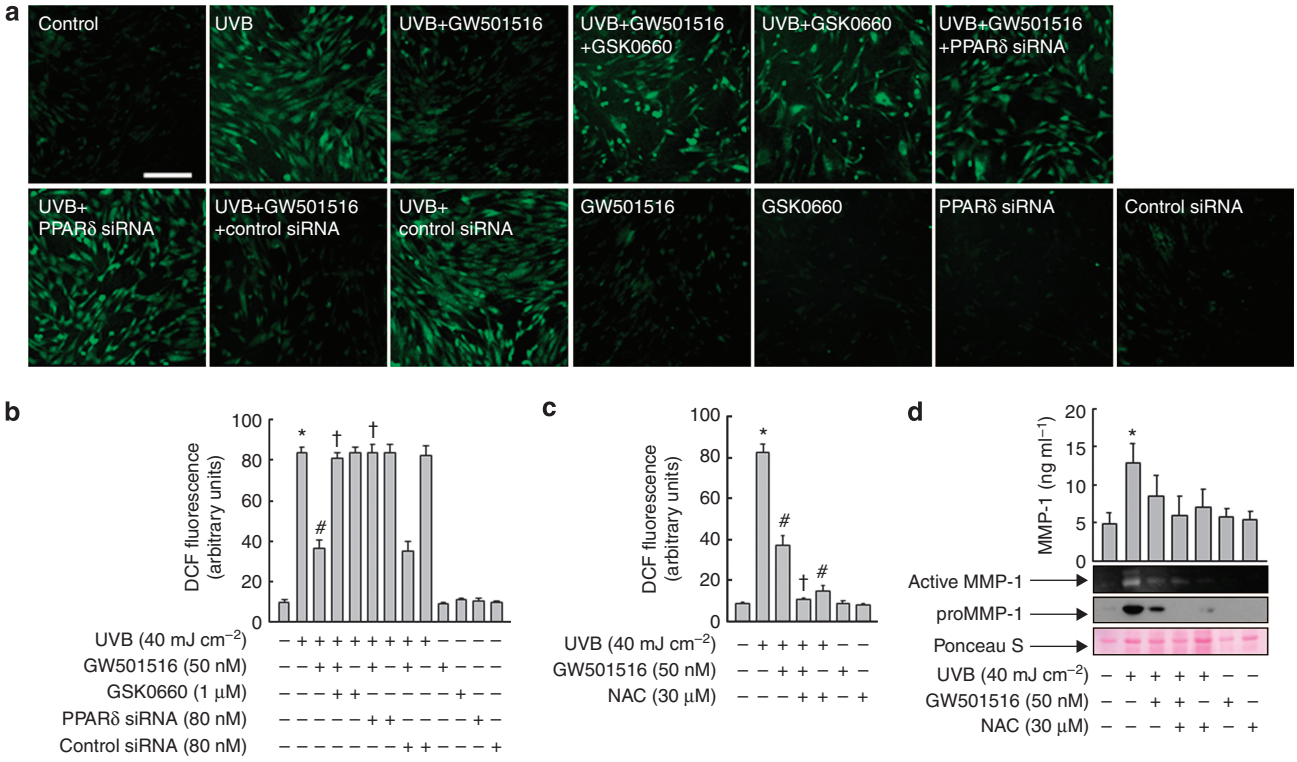

Figure 2. Peroxisome proliferator–activated receptor (PPAR) δ suppresses UVB-induced reactive oxygen species (ROS) generation. Cells transfected with or without indicated small interfering RNA (siRNA) were pretreated with GSK0660 or *N*-acetyl-L-cysteine (NAC) for 30 min and then treated with GW501516 for 24 hours. After exposure to UVB, the cells were incubated for 30 minutes and then intracellular ROS levels were detected by (**a**) confocal laser fluorescence microscopy and (**b**, **c**) quantified. (**d**) Cells pretreated with NAC for 30 minutes were treated with GW501516 for 24 hours and exposed to UVB. After incubation for 48 hours, conditioned media were subjected to ELISA, zymography, or immunoblot analysis. Results are expressed as the means ± SD ($n = 3$ or 5). $^{*}P < 0.01$ versus untreated group; $^{\#}P < 0.01$ versus UVB-exposed group; $^{\dagger}P < 0.01$ versus UVB plus GW501516-treated group. MMP-1, matrix metalloproteinase 1.

generation, MMP-1 secretion, comparable to the effect of GW501516 (Figure 2c and d), suggesting the involvement of ROS in the MMP-1 secretion induced by UVB irradiation.

PPARδ inhibits the UVB-induced activation of JNK

As ROS act as second messengers that activate the mitogen-activated protein kinase (MAPK) family (McCubrey *et al.*, 2006), we analyzed the involvement of the MAPK pathway in UVB-induced secretion of MMP-1. In HDFs exposed to UVB, three MAPK cascades were activated soon after UVB irradiation (Figure 3a). Among these pathways, ligand-activated PPARδ markedly inhibited the induction of JNK and p38 phosphorylation by UVB, but did not inhibit induction of the ERK pathway (Figure 3b).

To elucidate the signaling pathways involved in UVB-induced MMP-1 secretion, we examined the effects of specific inhibitors of the three MAPK cascades in HDFs exposed to UVB. As shown in Figure 3c, the UVB-induced increase in MMP-1 secretion was significantly reduced in the presence of SP600125, an inhibitor of the JNK pathway, and, to a lesser extent, PD98059 (an inhibitor of the ERK pathway) or SB203580 (an inhibitor of the p38 pathway). These results indicate that, although GW501516 inhibits both JNK and p38 signaling pathways, the JNK-mediated signaling pathway alone is involved in PPARδ-mediated blocking of MMP-1 secretion induced by UVB irradiation. In addition, the JNK inhibitor SP600125 reduced the secretion of MMP-1 following UVB irradiation to a similar extent to GW501516 (Figure 3d). Treatment with both GW501516 and SP600125, however, did not produce different results from treatment with GW501516 alone, indicating that the action of PPARδ in the inhibition of MMP-1 secretion is mediated through the JNK signaling pathway. In contrast, GW501516 did not affect the NF-κB and AP-1 signaling cascades activated by UVB irradiation, suggesting that NF-κB and AP-1 are not involved in the PPARδ-mediated inhibition of MMP-1 secretion (Supplementary Figure S4 online).

MKP-7 is stabilized by PPARδ

To investigate the molecular mechanism underlying the PPARδ-mediated inhibition of JNK phosphorylation in UVB-irradiated HDFs, we analyzed the levels of MKP-7, a JNK-specific phosphatase that negatively regulates the JNK signaling pathway (Tanoue *et al.*, 2001). As shown in Figure 4a and b, treatment with GW501516 markedly suppressed the UVB-induced phosphorylation levels of JNK in a dose-dependent manner. In contrast, the GW501516-mediated increase in MKP-7 protein was correlated with a decline in phosphorylated JNK. These data suggest that ligand-activated PPARδ regulates the activity of JNK at least in part by modulating MKP-7 levels.

To clarify further the molecular mechanism involved in the GW501516-mediated modulation of MKP-7 levels, we

determined whether the activation of PPARδ induces the expression of MKP-7 at a transcriptional level. In the presence of GW501516, the levels of MKP-7 transcript did not change, suggesting that PPARδ regulates MKP-7 levels posttranscriptionally (Supplementary Figure S5a and b online). Next, we examined the effects of GW501516 on the stability of MKP-7 transcripts in UVB-exposed HDFs, using actinomycin D to shut down RNA synthesis. At early times, the level of MKP-7 transcript decreased more rapidly in UVB-exposed HDFs than in control cells (unirradiated, treated with actinomycin D alone). In contrast, in UVB-irradiated cells treated with GW501516, MKP-7 messenger RNA (mRNA) levels were maintained as long as in the control cells (Figure 4c and d). These data suggest that ligand-activated PPARδ modulates the JNK signaling pathway by stabilizing MKP-7 mRNA.

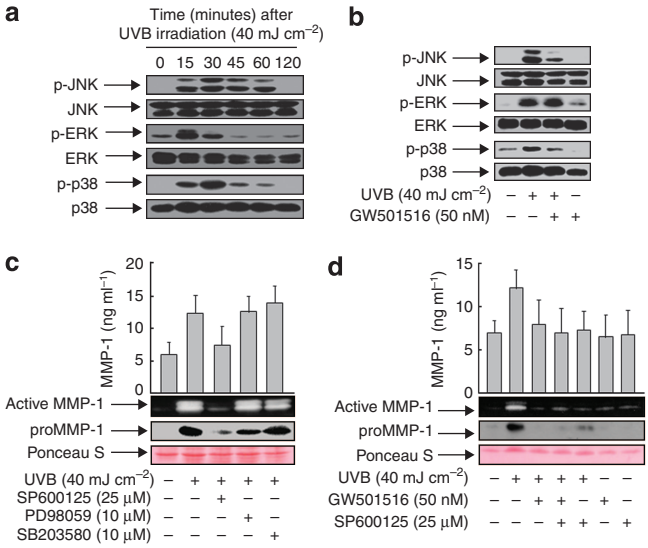

Figure 3. c-Jun N-terminal kinase (JNK) signaling cascade is responsible for the peroxisome proliferator–activated receptor-mediated inhibition of matrix metalloproteinase (MMP)-1 in human dermal fibroblasts (HDFs) exposed to UVB. (**a**) HDFs were exposed to UVB for the indicated times. (**b**) Cells pretreated with GW501516 for 24 hours were exposed to UVB for 30 minutes. An aliquot of protein was immunoblotted with activation-specific antibodies, and parallel immunoblots were analyzed for total kinase levels. (**c**) Cells were pretreated with SP600125, PD98059, or SB203580 for 30 minutes and then exposed to UVB. (**d**) Cells pretreated with SP600125 for 30 minutes were treated with GW501516 for 24 hours and then exposed to UVB. After incubation for 48 hours, conditioned media were subjected to ELISA, zymography, or immunoblot analysis. Results shown are representative of three or four independent experiments. ERK, extracellular signal–regulated kinase.

PPARδ prevents UVB-induced degradation of collagen I and III

To verify the functional significance of PPARδ-mediated inhibition of MMP-1 secretion in HDFs exposed to UVB, we examined the effects of GW501516 on UVB-induced breakdown of collagen. UVB irradiation reduces the levels of collagen I and III; as shown in Figure 5a, GW501516 reversed this suppression. This effect of GW501516 on collagen levels was dose-dependent and associated with a decline in the levels of MMP-1. Furthermore, treatment with NAC and SP600125 led to a recovery in collagen levels, comparable to that caused by GW501516 treatment (Figure 5b and c). These results suggest that in cells exposed to UVB, activation of PPARδ restores the levels of collagen I and III by inhibiting the secretion of MMP-1 through the modulation of ROS generation.

PPARδ prevents UVB-induced skin deformity in HR-1 hairless mice

Next, we examined the effects of GW501516 in HR-1 hairless mice exposed to UVB. Before animal experiments, we determined the dosage of GSK0660 to block the effect of GW501516 on the MMP-1 secretion in HDFs. GSK0660 of

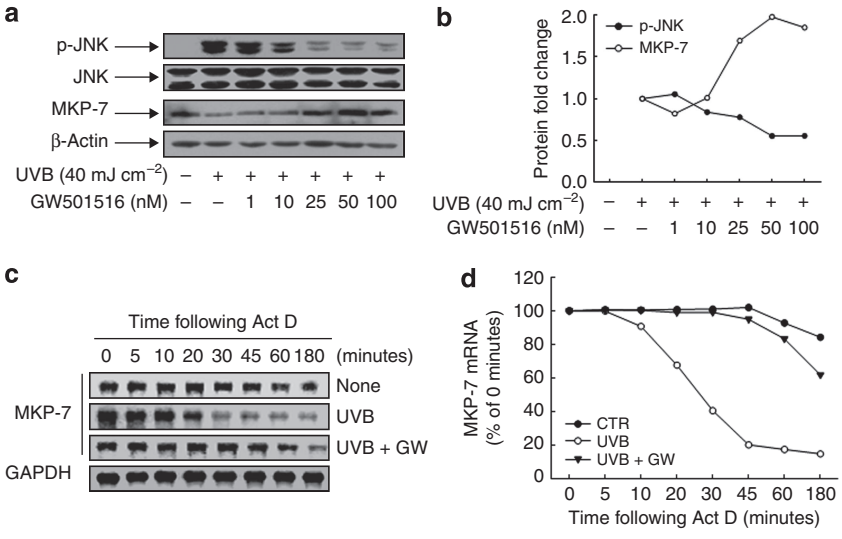

Figure 4. Peroxisome proliferator–activated receptor δ attenuates UVB-induced activation of c-Jun N-terminal kinase (JNK) through the stabilization of mitogen-activated protein kinase phosphatase (MKP)-7. Human dermal fibroblasts (HDFs) pretreated with indicated doses of GW501516 for 24 hours were exposed to UVB irradiation for 30 minutes. Cell extracts were separated by electrophoresis, (**a**) immunoblotted with the indicated antibodies, and (**b**) quantified. HDFs pretreated with 50 nM GW501516 (GW) for 24 hours were exposed to UVB (40 mJ cm^{-2}) for the indicated times in the presence of actinomycin D (Act D) (5 μg ml^{-1}). (**c**) Total messenger RNA was extracted and subjected to northern blot analysis. (**d**) The radioactivity of the signals was quantified using an image analyzer; data are expressed as ratios of MKP-7 to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), normalized against the ratio at time zero.

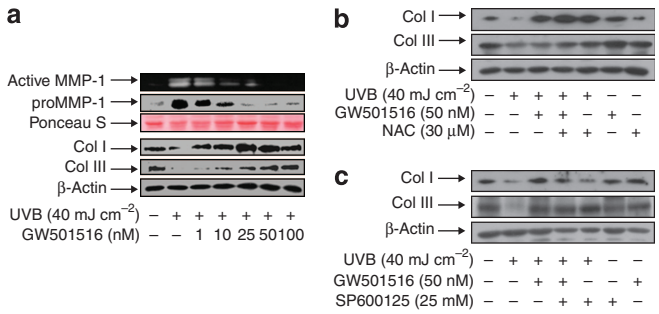

Figure 5. Peroxisome proliferator–activated receptor δ prevents breakdown of type I and III collagen in UVB-irradiated human dermal fibroblasts (HDFs). HDFs pretreated with a range of GW501516 for 24 hours were exposed to UVB irradiation. Following incubation for 48 hours, conditioned media or cell extracts were harvested and analyzed by (**a**) zymography or immunoblot analysis. Cells pretreated with (**b**) *N*-acetyl-L-cysteine (NAC) or (**c**) SP600125 for 30 minutes were incubated with GW501516 for 24 hours. Following exposure to UVB for 30 minutes, the cells were incubated for 48 hours. Cell extracts were analyzed by western blotting with the indicated antibodies. Representative images from three independent experiments are shown. MMP-1, matrix metalloproteinase 1.

10-fold lower molar ratio almost completely abolished the GW501516-mediated effects on the JNK activation and MMP-1 secretion induced by UVB irradiation (Supplementary Figure S6 online). Consistent with results from the *in vitro* studies, UVB irradiation increased wrinkle formation and epidermal/dermal thickness in the dorsal skin of hairless mice, as determined by the wrinkle formation assay and hematoxylin and eosin staining, respectively (Figure 6a and b). In contrast, administration of GW501516 significantly attenuated UVB-induced wrinkle formation and skin thickness. These effects of GW501516 were significantly reversed in mice pretreated with GSK0660. However, significant difference in the body weight among the experimental groups was not observed (Supplementary Figure S7 online). These results suggest that UVB irradiation induces morphological and histological changes in skin, and that PPARδ is an endogenous anti-aging molecule that can be activated and therapeutically targeted by its agonistic ligand, thereby preventing UVB-mediated skin damage.

In order to identify the molecules involved in the suppression of UVB-induced skin damage by PPARδ, we examined the levels of MMP-1, collagens, MKP-7, and JNK phosphorylation. When hairless mice were irradiated with UVB, MMP-1 and JNK phosphorylation was upregulated, with a concomitant decrease in the levels of MKP-7, collagen I and III. However, administration of GW501516 caused a marked decrease in the MMP-1 expression and JNK phosphorylation, with a corresponding increase in the levels of MKP-7, collagen I and III (Figure 6c–e). These results suggest that ligand-activated PPARδ downregulates JNK phosphorylation and MMP-1 expression, thereby increasing the levels of MKP-7 and collagens, ultimately preventing or suppressing UVB-induced skin disorders.

DISCUSSION

In this study, we demonstrated that the ligand-activated PPARδ attenuates the UVB-induced secretion of MMP-1 by reducing intracellular ROS generation. This effect of PPARδ is mediated by MKP-7, a phosphatase that regulates the JNK signaling cascades (Tanoue *et al.*, 2001). Our analysis of MAP kinases demonstrated that JNK takes part in the PPARδ-mediated inhibition of MMP-1 secretion induced by UVB irradiation. Furthermore, we showed that administration of a specific ligand for PPARδ, GW501516, inhibits the MMP-1 expression and JNK phosphorylation, increasing the levels of MKP-7, collagen I and III, and ultimately preventing UVB-induced skin deformity in hairless mice.

The increase of MMP activity in the skin is a primary causal factor in the progression of aging-related change in skin tissue structure and function. In our experiments, ligand-activated PPARδ markedly inhibited the UVB-induced secretion of MMP-1, with a concomitant increase in the levels of collagen I and III. This finding is in line with a previous study in which PPARα and PPARγ, two other members of the PPAR family, suppressed transforming growth factor-α-induced MMP-9 expression in human keratinocytes (Meissner *et al.*, 2011); PPARδ has also been suggested to be functionally important in suppressing MMP expression in that system (Meissner *et al.*, 2011). On the basis of these studies, we postulate that nuclear factors of the PPAR family modulate the balance between MMP activity and collagen expression in order to maintain skin homeostasis. As the increased expression of MMP-1 observed in UV-irradiated human skin leads to wrinkle formation and manifestations of aging (Brennan *et al.*, 2003), the findings we present here provide insights into the primary role of PPARδ as an anti-aging factor, specifically in the context of skin photoaging.

Upon exposure to UV irradiation, different MAPKs mediate the signal transduction pathways that lead to photoaging (Fisher *et al.*, 1998). In HDFs, the activation of the JNK and p38 MAPK cascades has a key role in UVB-induced photoaging (Bae *et al.*, 2008), whereas ERK, JNK, and p38 MAPK are all involved in UVB-induced photoaging in hairless mice (Afaq *et al.*, 2003; Sharma *et al.*, 2007). In this study, three MAP kinases were activated by UVB irradiation. UVB-mediated activation of JNK and p38, but not ERK, was markedly suppressed in the presence of GW501516. Although both JNK and p38 MAPK pathways are suppressed by PPARδ activation, they did not participate equally in the PPARδ-mediated inhibition of MMP-1 secretion in HDFs exposed to UVB. Inhibition of the JNK signaling cascade significantly reduced UVB-induced MMP-1 secretion, whereas a p38 MAPK inhibitor, SB203580, had no effect. Accordingly, the identity of the MAPK signaling cascade that mediates the MMP-1 secretion in UVB-exposed HDFs may be context-specific. Further studies will therefore be necessary to clarify the role of individual MAPK signal cascades in UVB-induced MMP-1 secretion.

Stabilization of the MKP-7 transcript by PPARδ is a key event in the blockade of UVB-induced secretion of MMP-1 by GW501516. The dual-specificity protein phosphatase MKP-7, a negative regulator of the MAPK signaling pathway, specifically inactivates JNK signaling (Masuda *et al.*, 2003, 2010). A previous study reported that activation of ERK induces phosphorylation of MKP-7 at Ser-446, which in turn

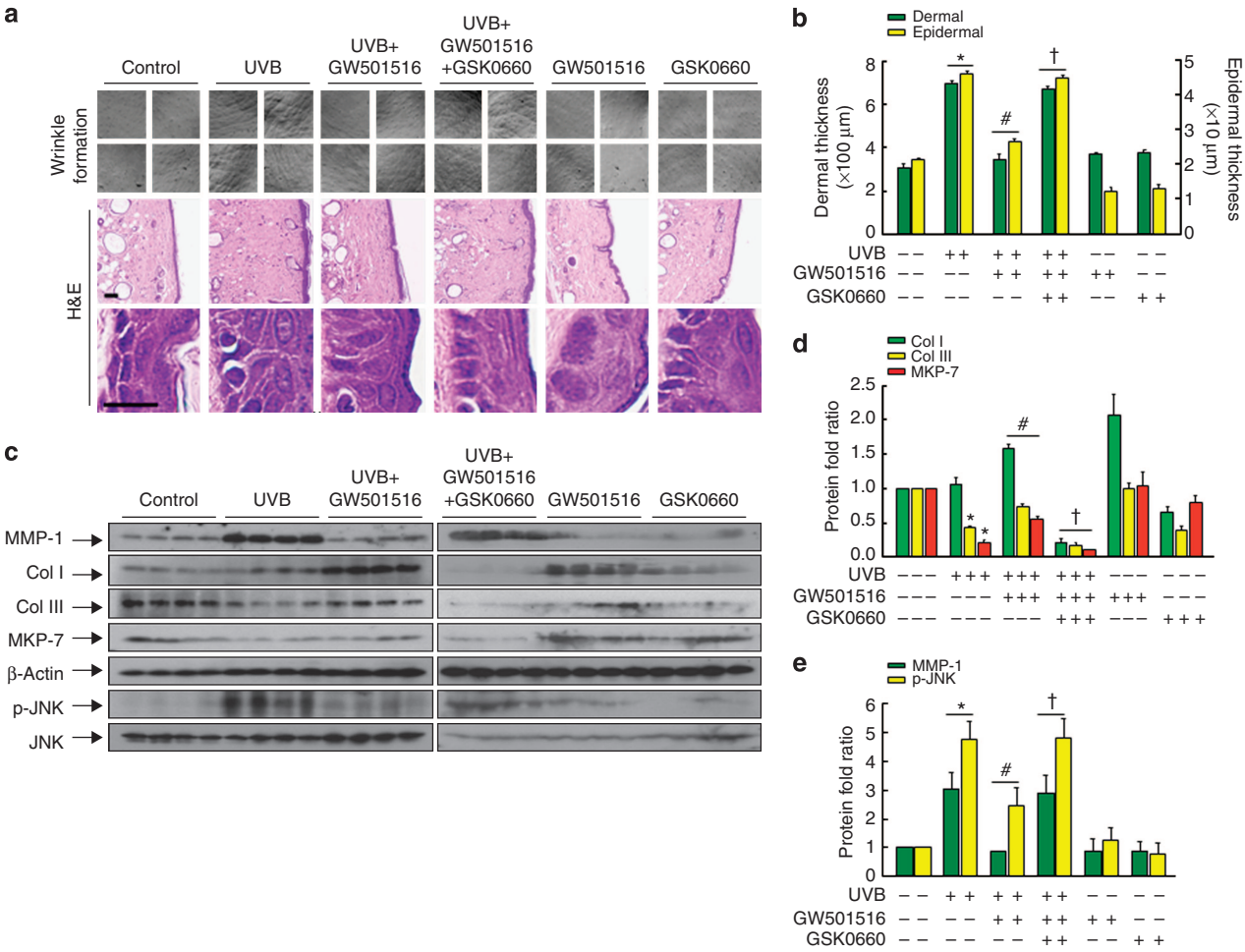

Figure 6. Peroxisome proliferator–activated receptor δ prevents UVB-induced skin deformity in HR-1 hairless mice. (**a**–**e**) Representative cross-sections of the dorsal skin of UVB-exposed HR-1 hairless mice treated with or without GSK0660 and/or GW501516. Wrinkle formation was detected by silicon replica assay (**a**). Paraffin tissue sections were subjected to hematoxylin and eosin (H&E) staining and quantitative analysis of epidermal and dermal thickness was performed (**a**, **b**). Bars = 100 μm. (**c**) Tissue extracts of dorsal skin from mice were subjected to immunoblot analysis. (**d**, **e**) Relative band intensities were quantified using an image analyzer and represented as a fold change relative to the untreated control. $^{*}P<0.01$ versus untreated group; $^{\#}P<0.01$ versus UVB-exposed group; $^{\dagger}P<0.01$ versus UVB plus GW501516-treated group. JNK, c-Jun N-terminal kinase; MKP, mitogen-activated protein kinase phosphatase; MMP-1, matrix metalloproteinase 1.

stabilizes the MKP-7 transcript (Katagiri *et al.*, 2005). However, the posttranslational modification of MKP-7 is poorly understood. PPARδ was originally identified as a transcription factor that forms heterodimers with RXR and induces the transcription of genes through the PPAR response element, located in target gene promoters (Tugwood *et al.*, 1992). However, the activity of PPARδ in stabilizing MKP-7 mRNA is not strictly consistent with its identity as a transcription factor (Sertznig *et al.*, 2008), suggesting that the posttranscriptional regulation of MKP-7 transcript by PPARδ results from a heretofore uncharacterized biochemical activity (Han *et al.*, 2008). As our results show that GW501516 does not affect the ERK phosphorylation induced by UVB, it is possible that ERK-mediated phosphorylation of MKP-7 protein may be responsible for the stabilization of MKP-7 transcript by PPARδ in cells exposed to UVB.

In line with our findings in cultured HDFs, the administration of GW501516 significantly attenuated UVB-induced skin deformity *in vivo*. Upon exposure to UVB, reduced MMP-1 secretion and increased levels of collagen I and III were observed in the dorsal skin of HR-1 hairless mice treated with GW501516. Concomitantly, wrinkle formation and epidermal/dermal thickness were significantly suppressed in these mice. Several environmental factors involved in photoaging have been shown to enhance MMP-1 activity (Brenneisen *et al.*, 2002; Brennan *et al.*, 2003). Increased expression and activity of MMP-1 are observed in aged or photoaged human skin lesions, but not in normal skin (Dong *et al.*, 2008; Matsui *et al.*, 2009). Given the fact that inhibition of MMP-1 expression reduces the progression of photoaging in primary HDFs (Yang *et al.*, 2011; Lee *et al.*, 2012), sustained secretion of MMP-1 may promote cellular photoaging through the breakdown of collagenous proteins in skin exposed to UV. In this context, PPARδ may serve as an anti-aging mediator in UV damage–related skin alterations such as wrinkle formation (Kambayashi *et al.*, 2001).

In conclusion, we have demonstrated that GW501516-activated PPARδ regulates UVB-induced photoaging in HDFs by inhibiting secretion of MMP-1. This inhibition occurs through the modulation of the MKP-7-mediated JNK signaling cascade, which thereby suppresses the breakdown of collagenous proteins. Our data advance our understanding of the molecular mechanisms underlying the anti-photoaging effects of PPARδ, and provide an insight regarding a therapeutic target for treatment of skin disorders.

MATERIALS AND METHODS

Cell culture and UVB irradiation

Primary HDFs (foreskin) were obtained from Welskin (Seoul, Korea) and were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 in the DMEM containing antibiotics and 10% fetal bovine serum. UVB irradiation was performed on serum-starved monolayer cultures using an FS20 lamp (National Biological, Twinsburg, OH).

Cell viability assay

Cell viability was determined according to a previously described method (Kang *et al.*, 2007).

Determination of MMP-1 secretion

Aliquots of conditioned culture media from equal numbers of HDFs were used to determine the relative amounts of MMP-1 secreted into the culture media. Equal volumes of conditioned culture media were precipitated with 80% ice-cold acetone and resuspended in SDS-PAGE sample buffer, and then subjected to zymography or immunoblot analysis. The Ponceau S staining was used to confirm equal loading.

Gene silencing with siRNA

HDFs were transfected with control or human PPARδ siRNA (Ambion, Austin, TX) in serum-free medium using Welfect-Q (WelGENE, Daegu, Korea) as described previously (Ham *et al.*, 2012).

Enzyme-linked immunosorbent assays

To determine MMP-1 in the conditioned medium of HDFs, the ELISA kit was used according to the manufacturer's instructions (Abcam, Cambridge, UK).

Gelatin zymography

Equal volumes of conditioned media were fractionated on 8% SDS-PAGE gel containing 2 mg ml^{-1} gelatin and stained in 1% Coomassie blue as described previously (Miyazaki *et al.*, 1990).

Western blot analysis

Aliquots of cell lysates or conditioned media were subjected to immunoblot analysis as described previously (Ham *et al.*, 2012).

Northern blot analysis

Expression of MKP-7 mRNA was determined according to the method described previously (Ham *et al.*, 2012). The complementary DNA probe was generated by PCR using primers specific for nucleotides 678–1,077 of human MKP-7.

Measurement of intracellular ROS

To assess the levels of intracellular ROS, HDFs were incubated with 10 μM H_2DCF-DA (Calbiochem, La Jolla, CA), a fluorescent probe, for 30 min at 37 °C, and then green fluorescence corresponding to the levels of intracellular ROS was detected through a 520-nm long-pass filter on an Olympus FV-1000 laser fluorescence microscope (Tokyo, Japan).

Animal study

All animal studies were approved by the Institutional Animal Care Committee of Konkuk University. Six-week-old hairless mice (HR-1) were obtained from Japan SLC (Hamamatsu, Japan). After injection with 1 mg kg^{-1} GSK0660 (Tocris Bioscience, Bristol, UK) for 30 min, mice were intraperitoneally injected with 10 mg kg^{-1} GW501516 (Enzo Life Science, Farmingdale, NY) and exposed to UVB radiation 1 hour later (Jung *et al.*, 2010). The injection of GSK0660 and/or GW501516 and UVB irradiation was applied to the mice three times a week for 8 weeks. UVB irradiation (100 J m^{-2}) was performed as described previously (Kim *et al.*, 2004). After 8 weeks, skin surface replicas were prepared by applying silicon (Dentkist, Seoul, Korea) to dorsal skin and mice were killed; squares of the dorsal skin (ca. 1 mm × 1 mm) were excised from each mouse and cut in half for histological examination (paraffin section: 4 μm) and protein analysis (Sato *et al.*, 1992). Dorsal skin sections were routinely stained with hematoxylin and esosin.

Statistical analysis

Data are expressed as means ± SD or SE. Statistical significance was determined by the Student's *t*-test or analysis of variance with a *post hoc* Bonferroni test. A value of $P<0.05$ was considered statistically significant.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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